Resistance to *Spodoptera frugiperda* (Lepidoptera: Noctuidae) and *Euxesta stigmatias* (Diptera: Ulidiidae) in Sweet Corn Derived from Exogenous and Endogenous Genetic Systems

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J. Econ. Entomol. 100(6): 1887–1895 (2007)

ABSTRACT Field trials using *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae) and *Euxesta stigmatias* Loew (Diptera: Ulidiidae) were conducted to evaluate resistance and potential damage interactions between these two primary corn, *Zea mays* L., pests against Lepidoptera-resistant corn varieties derived from both endogenous and exogenous sources. The endogenous source of resistance was maysin, a C-glycosyl flavone produced in high concentrations in varieties 'Zapalote Chico 2451′ and 'Zapalote Chico *sh2*'. The exogenous resistance source was the *Bacillus thuringiensis* (Bt)11 gene that expresses CryIA(b) insecticidal protein found in 'Attribute GSS-0966′. Damage by the two pests was compared among these resistant varieties and the susceptible 'Primetime'. Single-species tests determined that the Zapalote Chico varieties and GSS-0966 effectively reduced *S. frugiperda* larval damage compared with Primetime. *E. stigmatias* larval damage was less in the Zapalote Chico varieties than the other varieties in single-species tests. *E. stigmatias* damage was greater on *S. frugiperda*-infested versus *S. frugiperda*-excluded ears. Ears with *S. frugiperda* damage to husk, silk and kernels had greater *E. stigmatias* damage than ears with less *S. frugiperda* damage. Reversed phase high-performance liquid chromatography analysis of nonpollinated corn silk collected from field plots determined that isoorientin, maysin, and apimaysin plus 3′-methoxymaysin concentrations followed the order Zapalote Chico *sh*2 > Zapalote Chico 2451 > Attribute GSS-0966 = Primetime. Chlorogenic acid concentrations were greatest in Zapalote Chico 2451. The two high maysin Zapalote Chico varieties did as well against fall armyworm as the Bt-enhanced GSS-0966, and they outperformed GSS-0966 against *E. stigmatias*.

KEY WORDS Zapalote Chico, Primetime, fall armyworm, maysin

Resistance in field corn, *Zea mays* L., to lepidopterous pests has significantly advanced during the past 25 yr. Various types of germplasm have been released with resistance to several Lepidoptera, including two Crambidae, *Diatraea grandiosella* Dyar (southwestern corn borer) and *Ostrinia nubilalis* (Hübner) (European corn borer), and two Noctuidae, *Helicoverpa zea* (Boddie) (corn earworm) and *Spodoptera frugiperda* (J. E. Smith) (fall armyworm) (Scott and Davis 1981a, 1981b; Williams and Davis 1980, 1982, 1984; Williams et al. 1990; Davis et al. 1993; Scully et al. 2000b; Widstrom et al. 2003). This work was advanced largely through the transfer of resistant endogenous genes through intermating of adapted germplasm with resistant land races. Examples of resistant characters associated with endogenous genes include increased levels of cysteine proteinase, cuticular lipids, hydroxamic acids, and C-glycosyl flavones. Cysteine proteinases build up in yellow-green leaf tissues within the corn whorl in response to insect feeding and can reduce insect growth by >60% (Pecan et al. 2000). Cuticular lipids on leaves of Lepidoptera-resistant field corn result in increased movement of larvae and larvae fed such leaves developed slower than on leaves where the lipids had been removed (Yang et al. 1991, 1993). Increased levels of hydroxamic acids, which can be induced by plant damage, provide resistance to several group of insects, including aphids [corn leaf aphid, *Rhopalosiphum maidis* (Fitch), Heteroptera: Aphididae; Bernasconi et al. 1998], beetle larvae (western corn rootworm, *Diabrotica virgifera virgifera* LeConte, Coleoptera: Chrysomelidae; Assabgui et al. 1995), and lepidopteran larvae (European corn borer; Klum and Robinson 1969). Corn silks with elevated

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levels of maysin, a C-glycosyl flavone introduced into field corn through crosses to 'Zapalote Chico' populations and other germplasm (Waiss et al. 1979), are negatively correlated with larval weight of *H. zea* and *S. frugiperda* (Wiseman et al. 1992a). Isoorientin (6′-C-glucosyl-luteolin), an analog of maysin previously referred to as 6-C-galactosyl-luteolin (Snook et al. 1994), in the corn inbred T218 is thought to produce responses similar to maysin in *H. zea* (Widstrom and Snook 1998).

Exogenous, resistant genetic material in the form of genes coding for production of *Bacillus thuringiensis* ssp. *kurstaki* (Btk) (Berliner) endotoxins [CryIA(a)] were introduced into field corn in 1992 (Koziel et al. 1993). These advances led to several options available for the commercial production of field and silage corn resistant to several important lepidopterous corn pests. Bt field corn was shown to be resistant to *O. nubilalis* first generation leaf feeding and second generation stalk tunneling (Armstrong et al. 1995). Leaf-feeding damage, larval growth rates, and survival for *S. frugiperda* and *D. grandiosella* were significantly less on Northrup King Company (now Novartus Seeds, Research Triangle Park, NC) Bt field corn hybrids than on resistant hybrids with resistance derived from cysteine proteinase (Williams et al. 1997). Research on host plant resistance to various insects of maize has primarily focused on lepidopterous insects that attack field corn. However, the same lepidopterous pests that attack field corn also attack sweet corn.

In 1994, we began work to breed sweet corn plants that would produce elevated levels of maysin to protect the ears against lepidopterous pests. Beginning with a population of lepidopteran-resistant Zapalote Chico field corn with an average maysin content of 0.39% silk fresh weight, our work produced a *sh2* sweet corn ('Zapalote Chico *sh2*') with even greater levels of maysin (0.97% silk fresh weight), which was released in 1999 (Scully et al. 2000b). A transgenic sweet corn containing a synthetic gene (Bt11 event) for production of a Btk insecticidal protein [CryIA(b)] was released and shown to have foliar and ear resistance to *H. zea* and *S. frugiperda* in 1998 (Lynch et al. 1999a).

Although production of corn resistant to lepidopterous pests can result in significantly reduced insecticide inputs, other pests attack corn. *Euxesta stigmatias* Loew (Diptera: Ulidiidae) causes significant damage to sweet corn throughout the year in southern Florida (Nuessly and Hentz 2004). Larvae from eggs deposited into the silk channel attack the silk, cob, and kernels, reducing kernel set or rendering the entire ear unmarketable. Although sweet corn varieties with the Bt11 event can significantly reduce damage from fall armyworm and corn earworm (Lynch et al. 1999b), they have no effect on *E. stigmatias* (G.S.N., unpublished data). Therefore, growers must continue to apply insecticides to protect against this fly pest even if they are growing corn varieties protected by the current Bt genes.

To fill this void, we conducted research to evaluate sweet and field corns for resistance to *E. stigmatias.* Field trials determined that there was a considerable range of damage to corn ears caused by this pest (Scully et al. 2000a). Varieties with resistance imparted by cysteine proteinases, cuticular waxes, or maysin showed lower levels of damage by *E. stigmatias* compared with other varieties. Upon the release of Zapalote Chico *sh2* (Scully et al. 2000b), there were now two sources of resistance to fall armyworm in sweet corn: endogenous genetic material (i.e., maysin based resistance) and exogenous genetic material [Cry1A(b)]. The purpose of this research was to evaluate these two resistant sweet corns, as well as a sweet corn susceptible to Lepidoptera and a resistant field corn with the greatest levels of maysin available at that time, for resistance to both *S. frugiperda* and *E. stigmatias.*

Materials and Methods

Two hybrids and two germplasm lines were tested for ear resistance to *S. frugiperda* and *E. stigmatias,* including 'Primetime' (yellow, super sweet corn, Rogers Brand, Syngenta Seed, Boise, ID), 'Attribute GSS-0966′ (yellow, *sh*2, Bt-enhanced super sweet corn, Rogers Brand, Syngenta Seed, Boise, ID), 'Zapalote Chico 2451′ (Widstrom et al. 2003) and Zapalote Chico *sh*2 (Scully et al. 2000b). Zapalote Chico 2451 is a high-maysin floury corn derived from an Oaxacan land race known as Gpo. 35, whereas Zapalote Chico *sh*2 is a high-maysin *sh*2 conversion of Zapalote Chico 2451 (Widstrom et al. 2003). These varieties were grown outdoors at the Everglades Research and Education Center (EREC) in aboveground, concrete-walled production bins (92.2 m in length, 0.77 m in width, and 0.68 m in depth [interior dimensions]) filled with Palm Beach soil mix (50% compost, 25% clean sand, 25% bark, Odum's, Loxahatchee, FL). A double row (5 cm apart) of each variety was planted in each bin. Seeds were planted 15.2 cm apart in rows spaced 61 cm on center. Plants within each bin (block in model) were thinned to four to six plants of each variety at the five-leaf stage. A complete fertilizer plus micronutrients was mixed with the soil before planting. Insecticides were applied weekly to Primetime and the two Zapalote Chico varieties to protect the plants from fall armyworms until ears emerged. Additional foliar (20-20-20 plus micronutrients, and Mn) and soil applied (ammonium nitrate) fertilizers were applied at label rates on a regular basis.

Three experiments were designed to measure for resistance to 1) *S. frugiperda,* 2) *E. stigmatias,* and 3) the interaction of *S. frugiperda* and *E. stigmatias.* The first two experiments were designed to assess resistance to a single insect pest by using a randomized complete block design. The third experiment assessed resistance to both pests jointly by using a split-plot experiment arranged in a randomized complete block design. All three experiments were repeated in each of three consecutive years (i.e., years in the model) with three to 10 replicates (blocks) each year. Damage to the ears by each insect was rated on different scales. Larval *S. frugiperda* damage was rated on a 0–3 scale (modified from Widstrom 1967): 0, no damage; 1, husk damage; 2, silk damage; and 3, kernel damage. Damage by *E. stigmatias* larvae was rated on 0–4 scale: 0, no damage; 1, silk damage; 2, silk plus top 1–25% of the ear with kernels damaged; 3, silk plus top 26–50% of ear with kernels damaged; and 4, silk plus >50% of ear with kernels damaged (Scully et al. 2002).

Single insect-species tests for *S. frugiperda* resistance were conducted by placing larvae in test plant ears within 48 h of silk emergence from ear tips. Four to five first or second instars were placed in the silk channel of each ear. Larvae were collected as needed from a sweet corn trap crop grown each season at EREC. Ears were covered with #218 shoot bags (Lawson Pollinating Bags, Northfield, IL) before silk emergence to protect ears from natural infestation by armyworms and other pests before artificial infestation. Bags were briefly removed to infest ears with armyworm larvae and then replaced over the ears for 3 d to aid in larval establishment. Natural populations of *E. stigmatias* heavily infested the ears in the first study year. Because our goal was to examine damage rates by *S. frugiperda* alone in these experiments, insecticides were applied during the second and third year studies to reduce infestation by the flies after *S. frugiperda* larvae were established in the ears and the bags removed. Stalks above and leaves below the ears were sprayed three times weekly for control of *E. stigmatias* adults by using label rates of permethrin (Pounce 25 WP, FMC Corporation, Philadelphia, PA) insecticide applied with a CO_2 pressurized backpack sprayer. Ears were examined and rated for damage by both insects 21 d after silk emergence.

Resistance to *E. stigmatias* was tested in single-species tests by caging field-collected flies on ears to increase the chances for larval infestation. Three female and three male *E. stigmatias* adults were placed on individual ears within 32 by 32 mesh bags made from bridal tulle material and held on the ear with a rubber band near the ear shank. Mesh size was large enough to allow pollen to reach silks growing within the bags, but small enough to prevent the flies from getting their heads stuck in the material while exploring the bags. Flies were collected with a sweep net from sweet corn trap fields grown each season at EREC. Captured flies were lightly anesthetized using CO_2, separated by sex, and then placed into vials for release within 3 h into the ear cages. Ears were examined 21 d after first silk emergence and rated for damage by both insects.

Joint resistance to *S. frugiperda* and *E. stigmatias* was evaluated in a split-plot experiment. The main plots were ears either artificially infested with *S. frugiperda* larvae (i.e., infested) or not infested (i.e., excluded). Subplots were the four tested varieties. Ears of all plants within a bin (block) were either protected from *S. frugiperda* infestation by using an insecticide or were infested within 48 h of silk emergence with field collected first and second stage *S. frugiperda* larvae as described above. Ears and leaves below the ears on the excluded main plot bins were treated twice weekly with label rates of insecticide to greatly reduce the chances for natural fall armyworm infestation. Thiodicarb (Larvin 3.2, Bayer Crop Science, Research Triangle Park, NC) was selected to protect the ears from Lepidoptera, because previous experience had shown that it was effective against armyworm larvae, but ineffective at killing *E. stigmatias* (Nuessly and Hentz 2004). Ears in the *S. frugiperda*-excluded main plot bins also were covered with shoot bags until the bags over ears in the infested main plot bins were removed. Naturally occurring populations of *E. stigmatias* adults were allowed to select and oviposit on all ears after establishment of *S. frugiperda* larvae in the infested main plots. Ears were examined 21 d after first silk emergence and rated for damage by both insects.

Additional plants of each variety were grown solely to determine the concentration of maysin and other C-glycosyl flavones compounds produced in corn silk. Plants were grown outdoors at the USDA–ARS Crop Protection and Management Research Unit, Tifton, GA, during two seasons. Each variety was planted in a single plot each year. Seeds were planted 0.3 m apart in two rows 3 m in length on 0.9-m centers. Ears were covered with shoot bags to prevent pollination before silk emergence. Three- to 5-d-old silks were excised from the tips of ears, placed within a cold box and transported immediately to the laboratory where they were weighed, submerged in 100% MeOH by variety and placed in a freezer at −24°C. Ten and five 30-g samples were collected from each variety during the first and second seasons, respectively. Chemical analysis of silks was performed at the USDA–ARS Richard Russell Research Center, Phytochemical Research Unit, Athens, GA. Concentrations of chlorogenic acid, isoorientin, maysin, apimaysin, and 3′-methoxymaysin were determined by reversed phase high-performance liquid chromatography (Snook et al. 1989, 1993) and expressed as percentage of fresh weight of corn silk.

Analysis. PROC MIXED (SAS Institute 2001) was used to analyze the insect damage rating data due to the presence of both fixed and random effects in the experimental designs. Variety, treatment (*S. frugiperda* included or excluded in split-plot experiment), year, and their interactions were modeled as fixed effects. Block, block × treatment (split-plot experiment), block × variety, block × variety × year, block × treatment × year (split-plot experiment) were specified as random effects (RANDOM statement). Analysis of variance (ANOVA) was conducted using the TYPE1 Method due to unequal sample sizes. SAS calculated the degrees of freedom using the Containment Method. The LSMEANS statement was used to generate standard errors for the means, and the PDIFF command was used to perform *t*-test comparisons of the least square means.

PROC GLM was used to analyze the fresh weight concentrations of the four flavenoid pathway compounds. Values for *F* were calculated using the type 1 sums of squares due to unequal sample sizes. The Ryan–Einot–Gabriel–Welsch multiple range test was used for post hoc means separation.

Table 1. Mean ± SEM *S. frugiperda* ear damage rating[a] by year and variety in *S. frugiperda* exposure experiments

Variety	Yr 1			Yr 2			Yr 3		
	n	Mean ± SEM		*n*	Mean ± SEM		*n*	Mean ± SEM	
Primetime	38	2.6 ± 0.3a		24	1.6 ± 0.3a		56	3.0 ± 0a	
GSS-0966	35	0.9 ± 0.3b		23	0.7 ± 0.2b		59	2.9 ± 0.1a	
Zapalote Chico 2451	25	1.4 ± 0.3b		23	0.8 ± 0.2b		56	2.3 ± 0.1b	
Zapalote Chico *sh*2	31	1.2 ± 0.3b		17	0.9 ± 0.3b		51	2.5 ± 0.1b	
ANOVA	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Variety	3, 16.321	19.69	<0.0001	3, 14.353	2.20	0.1319	3, 26.127	14.75	<0.0001
Block	5, 16.368	6.80	0.0024	5, 14.133	0.58	0.7181	9, 26.14	0.97	0.4789
Variety × block	15, 105	0.91	0.5545	15, 63	1.79	0.0567	27, 182	1.05	0.4019

ANOVA calculated using PROC MIXED (SAS Institute 2001). Means in the same column followed by different letters are significantly different ($P \leq 0.05$; PDIFF paired *t*-tests; [SAS Institute 2001]).

[a] *S. frugiperda* ear damage ratings: 0, no damage; 1, husk damage; 2, silk damage; and 3, kernel damage.

Results

***S. frugiperda* Experiments.** Ear damage by *S. frugiperda* in single-species tests over the 3-yr study was significantly affected by variety ($F = 36.83$; df = 3, 10.066; $P < 0.0001$). Initial analysis of the combined data also determined that *S. frugiperda* damage ratings were significantly affected ($F = 73.93$; df = 2, 47.827; $P < 0.0001$) by year, so the data were split by year for further analysis. Variety significantly affected fall armyworm damage ratings in two of the years (Table 1). Primetime ears had significantly ($P < 0.05$) greater damage ratings than the other three varieties in the first year (Table 1). The same pattern was observed in the second year when *t*-tests indicated significant differences among the varieties, but this effect was not significant in the ANOVA. Fall armyworm damage ratings for Primetime and GSS-0966 were equivalent in year 3 when both were significantly greater than the two Zapalote Chico varieties.

Although the experiment was designed to evaluate *S. frugiperda* damage alone, *E. stigmatias* did infest the ears in all 3 yr, particularly in the first year when insecticides were not applied to control the flies. *E. stigmatias* damage was significantly affected by variety ($F = 86.97$; df = 3, 10.066; $P < 0.0001$), year, ($F = 34.82$; df = 2, 40; $P < 0.0001$), and the variety × year interaction ($F = 2.92$; df = 6, 42.385; $P = 0.0178$). Analysis of the data by year found that variety significantly affected *E. stigmatias* damage in first and third years (Table 2). *E. stigmatias* pressure was greatest on ears in the first year with mean damage rates nearly twice as great on Primetime as on the other varieties. Fly damage on the two Zapalote Chico varieties was significantly less than on Primetime in two of the 3 yr. Insecticide treatments for *E. stigmatias* kept the damage levels below 1.0 in the second and third years of the single-species *S. frugiperda* tests.

***E. stigmatias* Experiments.** Variety significantly affected ($F = 13.70$; df = 3, 8.2531; $P = 0.0014$) *E. stigmatias* damage to corn ears in single-species experiments for fly resistance. Damage by *E. stigmatias* also was significantly affected by year ($F = 16.38$; df = 2, 18.734; $P < 0.0001$). Results from the analysis of the data by year again found that variety significantly affected fly damage ratings in each of the 3 yr (Table 3). Fly damage was significantly lower on Zapalote Chico 2451 than Primetime in all years, and both the high-maysin–producing breeding lines had significantly lower fly damage than the Bt- and non-Bt-producing hybrids in the second and third years. Ear infestation by *S. frugiperda* in these single-species *E. stigmatias* exposure experiments was low to nonexistent, with mean damage ratings <0.1 in all years.

Combined *S. frugiperda* and *E. stigmatias* Experiments. The split-plot experiment was designed to evaluate whether damage by *S. frugiperda* affected *E. stigmatias* ear damage and whether plant variety played a

Table 2. Mean ± SEM *E. stigmatias* ear damage rating[a] by year and variety in *S. frugiperda* exposure experiments

Variety	Yr 1			Yr 2			Yr 3		
	n	Mean ± SEM		*n*	Mean ± SEM		*n*	Mean ± SEM	
Primetime	38	3.4 ± 0.4a		24	0.9 ± 0.3a		56	0.6 ± 0.1a	
GSS-0966	35	1.8 ± 0.4b		23	0.8 ± 0.3a		59	0.6 ± 0.1a	
Zapalote Chico 2451	25	1.8 ± 0.5b		23	0.6 ± 0.3a		56	0.1 ± 0.1b	
Zapalote Chico *sh*2	31	1.3 ± 0.5b		17	0.2 ± 0.3a		51	0.1 ± 0.1b	
ANOVA	df	*F*	*P*	df	*F*	*P*	df	*F*	*P*
Variety	3, 15.773	14.59	<0.0001	3, 14.609	0.83	0.4979	3, 26.718	4.52	0.0109
Block	5, 15.8	8.17	0.0009	5, 14.219	1.26	0.3332	9, 26.352	2.81	0.0188
Variety × block	15, 105	1.55	0.1026	15, 63	1.99	0.0304	27, 182	1.40	0.1021

ANOVA calculated using PROC MIXED (SAS Institute 2001). Means in the same column followed by different letters are significantly different ($P \leq 0.05$; PDIFF paired *t*-tests [SAS Institute 2001]).

[a] *E. stigmatias* ear damage ratings: 0, no damage; 1, silk damage; 2, silk plus top 1–25% of the ear with kernels damaged; 3, silk plus top 26–50% of ear with kernels damaged; and 4, silk plus >50% of ear with kernels damaged.

Table 3. Mean ± SEM *E. stigmatias* ear damage rating[a] by variety and year in *E. stigmatias* exposure experiments where *S. frugiperda* were excluded

Variety	Yr 1				Yr 2				Yr 3			
	n	Mean ± SEM			n	Mean ± SEM			n	Mean ± SEM		
Primetime	12	1.6 ± 0.3a			21	3.1 ± 0.3a			30	2.2 ± 0.5a		
GSS-0966	12	0.8 ± 0.3ab			23	3.1 ± 0.3a			30	2.2 ± 0.5a		
Zapalote Chico 2451	13	0b			20	1.3 ± 0.3b			30	0.2 ± 0.5b		
Zapalote Chico *sh*2	11	1.0 ± 0.3ab			20	2.1 ± 0.3b			30	0.7 ± 0.5b		
ANOVA		df	F	P		df	F	P		df	F	P
Variety		3, 5.7678	7.69	0.0192		3, 5.7784	11.50	0.0075		3, 12	8.77	0.0024
Block		2, 5.8337	0.49	0.6335		2, 5.8054	1.65	0.2715		4, 12	3.61	0.0374
Variety × block		6, 36	0.56	0.7627		6, 72	0.74	0.6163		12, 100	4.15	<0.0001

ANOVA calculated using PROC MIXED (SAS Institute 2001). Means in the same column followed by different letters are significantly different ($P \leq 0.05$; PDIFF paired t-tests [SAS Institute 2001]).

[a] *E. stigmatias* ear damage ratings: 0, no damage; 1, silk damage; 2, silk plus top 1–25% of the ear with kernels damaged; 3, silk plus top 26–50% of ear with kernels damaged; and 4, silk plus >50% of ear with kernels damaged.

mitigating role in any such relationship. Damage to ears caused by fall armyworm ($F = 4.75$; df = 2, 7.6376; $P = 0.0457$) and *E. stigmatias* ($F = 174.30$; df = 2, 7.3498; $P < 0.0001$) in the split-plot experiments were significantly affected by study year, so the data were split by year for analysis. Fly larva damage to corn ears was significantly worse on ears infested with fall armyworm in the second and third years (Table 4) when naturally occurring *E. stigmatias* populations were the greatest. As expected, exposing ears to *S. frugiperda* also increased *S. frugiperda* damage ratings in all three years (Table 5). Plant variety again significantly affected both *E. stigmatias* and *S. frugiperda* damage rates in all three years (Tables 4 and 5). *E. stigmatias* and *S. frugiperda* damage were greatest on *S. frugiperda*-infested Primetime in the first year when naturally occurring fly populations were low. Fly larva damage was significantly less on both *S. frugiperda*-infested and -excluded Zapalote Chico 2451 and Zapalote Chico *sh*2 than on Primetime and GSS-0966 in the third year when fly populations were the greatest. However, even the maysin in the Zapalote Chico lines did not protect the ears against *E. stigmatias* in the *S. frugiperda*-infested ears in the second year when high temperatures resulted in lose and open silk channels due to elongated cobs. Primetime ears sustained the greatest *S. frugiperda* damage of all the varieties in all 3 yr. Fall armyworm damage on *S. frugiperda*-infested Zapalote Chico 2451 and Zapalote Chico *sh*2 were equivalent to or less than on GSS-0966 in all 3 yr.

The affect of the three levels of *S. frugiperda* damage rating on *E. stigmatias* damage were further analyzed using the combined data set from the split-plot experiments and from the first year *S. frugiperda* single-species tests (i.e., when ears were not protected from *E. stigmatias*). ANOVA was conducted using PROC MIXED (SAS Institute 2001) with *S. frugiperda* damage rating, plant variety and their interaction term as the fixed effects and block as a random effect. *S. frugiperda* damage rating ($F = 11.59$; df = 3, 481; $P < 0.0001$), plant variety ($F = 7.60$; df = 3, 481; $P < 0.0001$), and block ($F = 4.61$; df = 3, 481; $P = 0.0004$) had significant affects on *E. stigmatias* damage ratings, whereas the interaction term was not significant ($F = 1.23$; df = 5, 481; $P = 0.2724$). Fly damage was significantly greater on level 3 *S. frugiperda*-damaged ears (2.7 ± 0.2) than for levels 1 (1.7 ± 0.3) and 0 (1.4 ± 0.2), but equivalent to level 2 (1.7 ± 0.5).

Chemical Analysis of Silks. Variety significantly affected percentage of fresh weights of all four of the tested flavonoid pathway compounds (Table 6). Year had a significant affect on all compounds except for maysin concentration. Mean concentrations of chlorogenic acid and isorientin were greater in the second than in first year tests and apimaysin plus 3′-methoxymaysin concentration was greater in the first than in the second year. However, the arrangement of mean flavenoid pathway compound concentrations from least to greatest was the same in both years for all four compounds; so, the data are presented for the combined data set. Isoorientin, maysin and apimaysin plus 3′-methoxymaysin concentrations were significantly greater in Zapalote Chico *sh*2 than in Zapalote Chico 2451, and values for both varieties were significantly greater than for Primetime and GSS-0966. Chlorogenic acid concentrations were greater in Zapalote Chico 2451 than in the other varieties.

Discussion

The high-concentration maysin and the CryIA(b)-producing varieties provided equivalent control of *S. frugiperda* larvae introduced into ears in both the single- and two-species experiments (Tables 1 and 5). Previous studies also have shown the effectiveness of sweet corn enhanced with Btk endotoxins (Lynch et al. 1999b) and maysin (Wiseman et al. 1978) in the control of *H. zea* and *S. frugiperda.* Chlorogenic acid, 3′-methoxymaysin, apimaysin, maysin (Wiseman et al. 1992b), and isoorientin (Widstrom and Snook 1998) produced in the flavonoid and phenylpropanoid pathways in maize silks (Guo et al. 1999) have all shown negative correlations with *H. zea* and *S. frugiperda* weight gain. But the majority of the antibiotic effect in these previous studies was correlated with maysin and its analog isoorientin, due to greater concentrations of these compounds relative to the others. Weight reductions of 60 and >90% have been found for *S. frugiperda* larvae fed diets made with corn silks containing ≥0.2% and 0.7% fresh weights of maysin,

Table 4. Mean ± SEM *E. stigmatias* ear damage ratings[a] by year and variety for joint *S. frugiperda* and *E. stigmatias* exposure experiments

Treatment *S. frugiperda*	Variety	Yr 1				Yr 2				Yr 3			
		n		Mean ± SEM		*n*		Mean ± SEM		*n*		Mean ± SEM	
Excluded	Primetime	17		0.3 ± 0.2bc		16		2.4 ± 0.3bc		14		3.6 ± 0.3a	
	GSS-0966	18		0.4 ± 0.2bc		13		2.3 ± 0.3c		17		3.4 ± 0.3a	
	Zapalote Chico 2451	7		0c		23		1.7 ± 0.3cd		15		1.0 ± 0.3c	
	Zapalote Chico *sh2*	15		0c		11		1.2 ± 0.4d		15		1.5 ± 0.3b	
Infested	Primetime	18		1.4 ± 0.3a		21		3.9 ± 0.3a		16		3.8 ± 0.3a	
	GSS-0966	16		0.8 ± 0.2ab		23		3.9 ± 0.3a		14		3.6 ± 0.3a	
	Zapalote Chico 2451	8		0c		20		3.3 ± 0.3ab		17		1.2 ± 0.3bc	
	Zapalote Chico *sh2*	11		0c		14		3.4 ± 0.3a		15		1.5 ± 0.3b	
ANOVA	Effects		df	*F*	*P*		df	*F*	*P*		df	*F*	*P*
	Treatment		1, 1.7725	7.83	0.1227		1, 1.9663	53.09	0.0192		1, 1.815	117.41	0.0117
	Variety		3, 99.111	4.75	0.0039		3, 128.68	3.74	0.0128		3, 111	43.58	<0.0001
	Treatment × variety		3, 99.843	2.00	0.1191		3, 128.66	0.65	0.5818		3, 111	0.38	0.7695
	Block		2, 1.932	0.33	0.7517		2, 1.8991	1.16	0.4686		2, 2	28.05	0.0344
	Treatment × block		2, 98	0.86	0.4246		2, 128	1.29	0.2777		2, 111	0.01	0.9856

ANOVA calculated using PROC MIXED (SAS Institute 2001). Means in the same column followed by different letters are significantly different ($P \leq 0.05$; PDIFF paired *t*-tests [SAS Institute 2001]).
[a] *E. stigmatias* damage ratings: 0, no damage; 1, silk damage; 2, silk plus top 1–25% of the ear with kernels damaged; 3, silk plus top 26–50% of ear with kernels damaged; and 4, silk plus >50% of ear with kernels damaged.

Table 5. Mean ± SEM *S. frugiperda* ear damage ratings[a] by variety and year for joint *S. frugiperda* and *E. stigmatias* exposure experiments

Treatment *S. frugiperda*	Variety	Yr 1				Yr 2				Yr 3			
		n		Mean ± SEM		*n*		Mean ± SEM		*n*		Mean ± SEM	
Excluded	Primetime	17		0c		16		0.4 ± 0.2bc		14		0.2 ± 0.2c	
	GSS-0966	18		0.1 ± 0.2c		13		0c		17		0c	
	Zapalote Chico 2451	7		0c		23		0.1 ± 0.2c		15		0c	
	Zapalote Chico *sh2*	15		0c		11		0c		15		0c	
Infested	Primetime	18		2.6 ± 0.2a		21		2.3 ± 0.2a		16		2.6 ± 0.2a	
	GSS-0966	16		0.7 ± 0.2b		22		0.3 ± 0.2c		14		1.7 ± 0.2b	
	Zapalote Chico 2451	8		0.1 ± 0.2c		20		0.8 ± 0.2b		17		1.4 ± 0.2b	
	Zapalote Chico *sh2*	11		0.3 ± 0.2bc		14		0.4 ± 0.2bc		15		1.3 ± 0.2b	
ANOVA	Effects		df	*F*	*P*		df	*F*	*P*		df	*F*	*P*
	Treatment		1, 1.139	456.63	0.0201		1, 1.9324	39.75	0.0264		1, 1.8669	198.37	0.0065
	Variety		3, 98.286	20.83	<0.0001		3, 128.55	17.49	<0.0001		3, 112.44	5.60	0.0013
	Treatment × variety		3, 98.175	21.34	<0.0001		3, 128.47	6.55	0.0004		3, 112.93	2.59	0.0561
	Block		2, 1.7398	0.16	0.8657		2, 1.8382	1.65	0.3882		2, 1.9975	3.94	0.2025
	Treatment × block		2, 98	0.22	0.8028		2, 128	0.80	0.4514		2, 111	0.63	0.5339

ANOVA calculated using PROC MIXED (SAS Institute 2001). Means in the same column followed by different letters are significantly different ($P \leq 0.05$; PDIFF paired *t*-tests [SAS Institute 2001]).
[a] *S. frugiperda* damage ratings: 0, no damage; 1, husk damage; 2, silk damage; and 3, kernel damage.

Table 6. Mean ± SEM percentage fresh weight concentration of maysin and analogs in corn silk by variety

Variety	n	Chlorogenic acid		Isoorientin		Maysin		Apimaysin + 3′-methoxy-maysin	
Primetime	15	0.107 ± 0.012	b	0	c	0.017 ± 0.004	c	0.001 ± 0.001	c
GSS-0966	12	0.110 ± 0.009	b	0	c	0.015 ± 0.005	c	0	c
Zapalote Chico 2451	15	0.360 ± 0.062	a	0.200 ± 0.024	b	4.485 ± 0.431	b	0.755 ± 0.082	b
Zapalote Chico *sh2*	13	0.198 ± 0.045	b	0.449 ± 0.051	a	10.376 ± 1.350	a	1.934 ± 0.218	a
ANOVA	df	*F*	*P*	*F*	*P*	*F*	*P*	*F*	*P*
Variety	3, 47	16.74	<0.0001	64.71	<0.0001	52.40	<0.0001	109.33	<0.0001
Yr	1, 47	19.29	<0.0001	5.23	0.0267	3.28	0.0765	14.20	0.0005
Variety × yr	3, 47	7.58	0.0003	1.69	0.1818	1.11	0.3533	8.27	0.0002

ANOVA calculated using PROC GLM (SAS Institute 2001). Means in the same column followed by different letters are significantly different (α = 0.05; REGW multiple range test [SAS Institute 2001]).

respectively (Wiseman et al. 1992a). Widstrom and Snook (1998) determined that corn silk with a fresh weight of >2.0% isoorientin was sufficient for inhibiting *H. zea* larval growth. Mean fresh weight maysin concentrations for Zapalote Chico 2451 and Zapalote Chico *sh*2 varieties in our study (Table 6) were ≈5 and 11 times greater, respectively, than those noted by Wiseman et al. (1992a) to cause 90% weight reduction in *S. frugiperda* larvae. In addition to the antibiotic affects of maysin, Wiseman et al. (1983) determined in an early study that nonpreference played a role in the resistance of Zapalote Chico 2451 P(C3) to *H. zea.* Larvae placed on Zapalote Chico silks and those given the initial choice of Zapalote Chico or the susceptible 'Stowell's Evergreen' overwhelmingly (>80%) chose to feed on the latter variety in repeated tests. Relatively high maysin concentrations and nonpreference were likely responsible for the low *S. frugiperda* damage ratings in the two Zapalote Chico varieties in our experiment.

Varieties with high maysin concentrations provided moderate resistance to *E. stigmatias* in our trials, whereas the CryIA(b)-producing variety (i.e., GSS-0966) did not reduce damage by *E. stigmatias* larvae over the susceptible Primetime (Tables 3 and 4). The susceptibility of GSS-0966 and Primetime ears to economic injury by *E. stigmatias* also has been observed in numerous field trials and commercial fields grown in southern Florida from 1998 through 2003 (G.S.N., unpublished data). Silks of the two Zapalote Chico varieties turned brown in response to feeding by both *S. frugiperda* and *E. stigmatias*, whereas those of GSS-0966 and Primetime did not. Browning of silks in response to wounding is associated with oxidation of the antibiotic flavones to quinones (Byrne et al. 1996). Quinones bind to –SH and $-NH_2$ groups of free amino acids and proteins reducing their availability to the insect and thus inhibiting larval growth and development (Felton et al. 1989, Wiseman and Carpenter 1995). The results of our study suggest that the antibiotic characteristics of maysin and the other C-glycosyl flavonones in maize silks (Guo et al. 1999) may extend beyond the Lepidoptera to the Diptera.

Results of our tests also suggest that excessive feeding by lepidopteran larvae compromises the ability of varieties with high maysin concentrations to resist *E. stigmatias* feeding. The two Zapalote Chico varieties had significantly lower fly damage ratings than the other varieties in the single-species experiments and in the split-plot experiments where *S. frugiperda* larvae were excluded. But *E. stigmatias* damage ratings on these two varieties were significantly greater in the split-plot experiments for ears where *S. frugiperda* larvae had eaten through the silk and into the kernels (i.e., level 3 damage) than in those with *S. frugiperda* damage restricted to the husk and silk (i.e., levels 1 and 2 damage). These greater fly damage ratings in ears heavily damaged by *S. frugiperda* larvae were equivalent to or greater than the ratings for GSS-0966 and Primetime where *S. frugiperda* were excluded (Table 4). Wiseman and Snook (1995) found that maysin concentration decreased as silks were pollinated and grew older. They found no significant weight reduction in *H. zea* larvae fed diets with incorporated 10-d old pollinated silk from cultivars with high maysin compared with diets with silks from varieties with no maysin. Yet in our study *E. stigmatias* damage ratings at 21 d after first silk were significantly less on the two Zapalote Chico varieties than on the other two varieties, even in ears with minor *S. frugiperda* damage. It is possible that *S. frugiperda* larval tunnels through the silk channel, while frequently lightly packed with frass, may provide *E. stigmatias* larvae easier access to the cob and kernels than in ears without substantial silk channel damage. Rare plants with low initial maysin concentrations may also be responsible for the greater damage levels caused by both insects on some ears. *E. stigmatias* larvae, and adults and larvae of sap beetles, were more common in standard than in Bt-enhanced field corn [Mon810 event expressing Cry1A(b) endotoxin] in Georgia (Daly and Buntin 2005). They suggested that lower levels of kernel damage by *H. zea* on the Bt line made the ears less attractive to both groups of insects.

In conclusion, the high mayzin producing Zaplalote Chico breeding lines did as well as Bt-enhanced Attribute GSS-0966 in protecting ears against fall armyworm and out performed GSS-0966 in protecting corn ears against *E. stigmatias* damage. The results of this study provide further evidence that insect-resistant traits associated with endogenous genes can provide effective control of primary insect pests in agricultural systems.

Acknowledgments

The assistance of M. Brennan with statistical analysis is greatly appreciated. We thank D. Sistrunk and R. Innocent for technical assistance. R. Cherry. W. Overholt, and P. J. van Blokland provided helpful reviews of the manuscript. This research was supported by Illinois Foundation Seed, Florida Foundation Seed, Syngenta Seed, USDA–ARS–CPMRU, and by the Florida Agricultural Experiment Station. This report approved for publication as USDA–ARS Journal Series.

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Received 10 November 2005; accepted 15 August 2007.